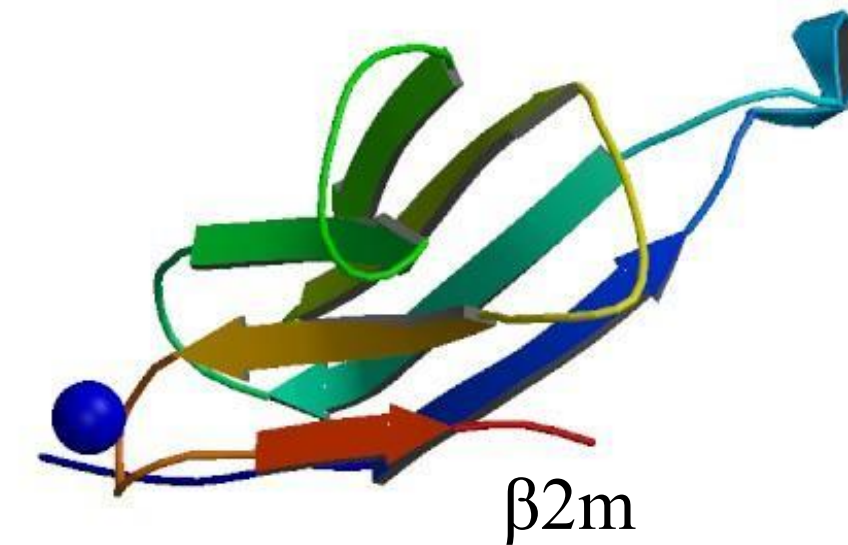


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BACKGROUND

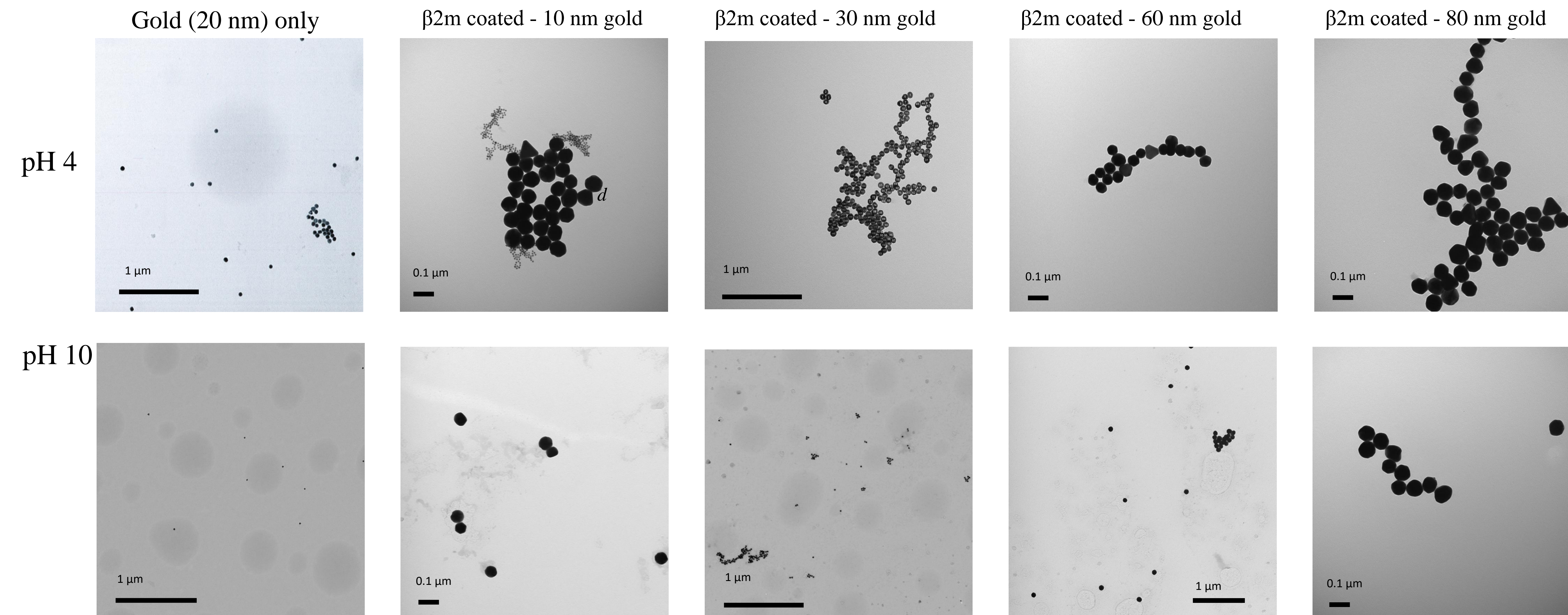
Human $\beta 2$ mircoglobulin ($\beta 2m$) is a protein of composed of 119 amino acids. It is an amyloidogenic protein that is a component of HLA (Human Leukocyte Antigen) class I, which widely exists in nucleated cell surfaces throughout the body. Among available amyloidogenic peptides, we chose $\beta 2m$ to study peptide networking using gold colloid aggregates, since $\beta 2m$ coated gold colloid aggregates form relatively less congested ensemble.



Our research group has been investigating the interfacial structure of amyloidogenic peptide over nano-scale gold metal surfaces, aiming to construct an intermediate structure of fibrillogenesis. The folded structure is supported over a nano-gold surface at basic conditions, while the unfolded structure is constructed at an acidic conditions and creates a network with other peptides. This network creates the aggregates which can be easily observed by TEM (Transmission Electron Microscopy).

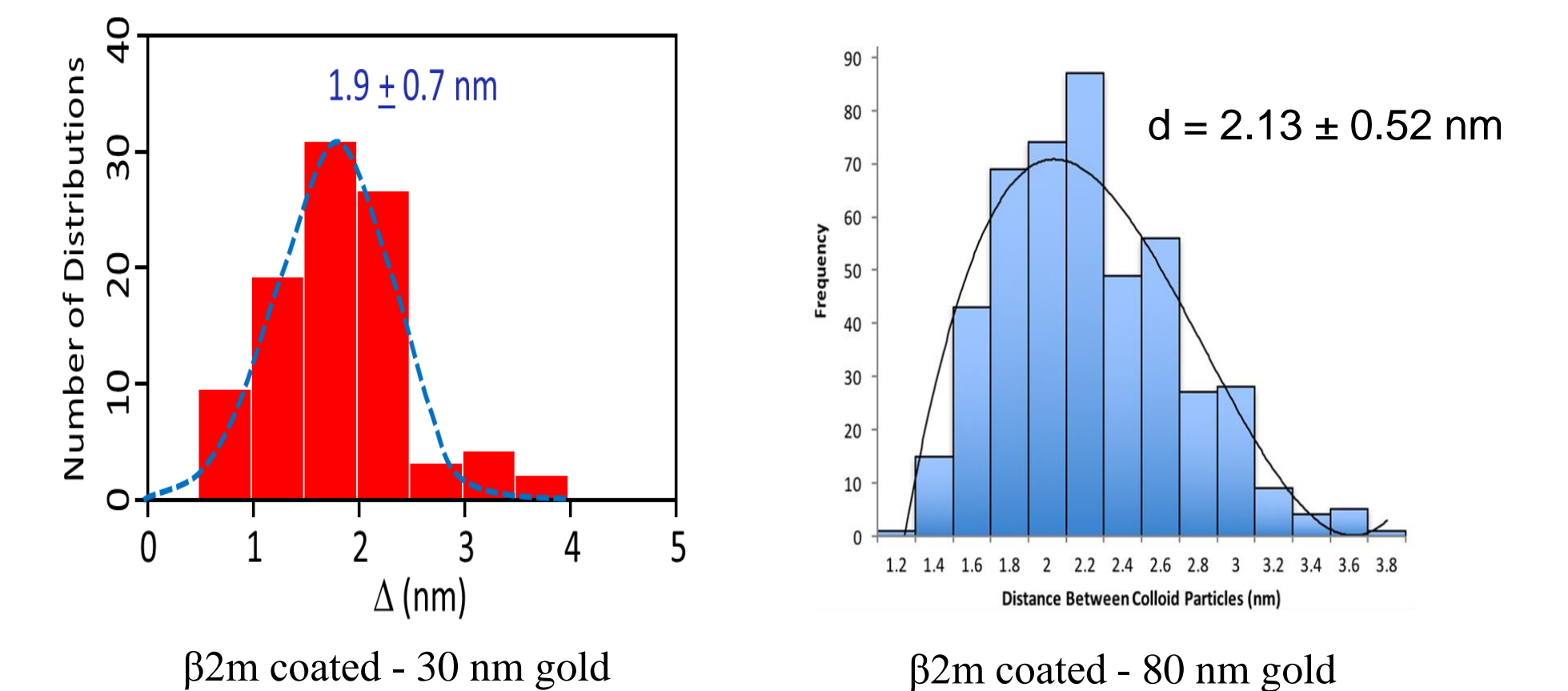
TEM IMAGES

TEM samples were prepared with $\beta 2m$ peptide and 10 nm, 30 nm, 60 nm, and 80 nm gold colloids. Hydrochloric acid and sodium hydroxide were added to change the pH between pH 10 and pH 4. Samples were plated onto copper Fomvar grids for inspection under the Morgagni Transmission Electron Microscope (TEM). The images were taken under 71,000x magnification using a model XR-40 four megapixel CCD digital camera.



RESULTS

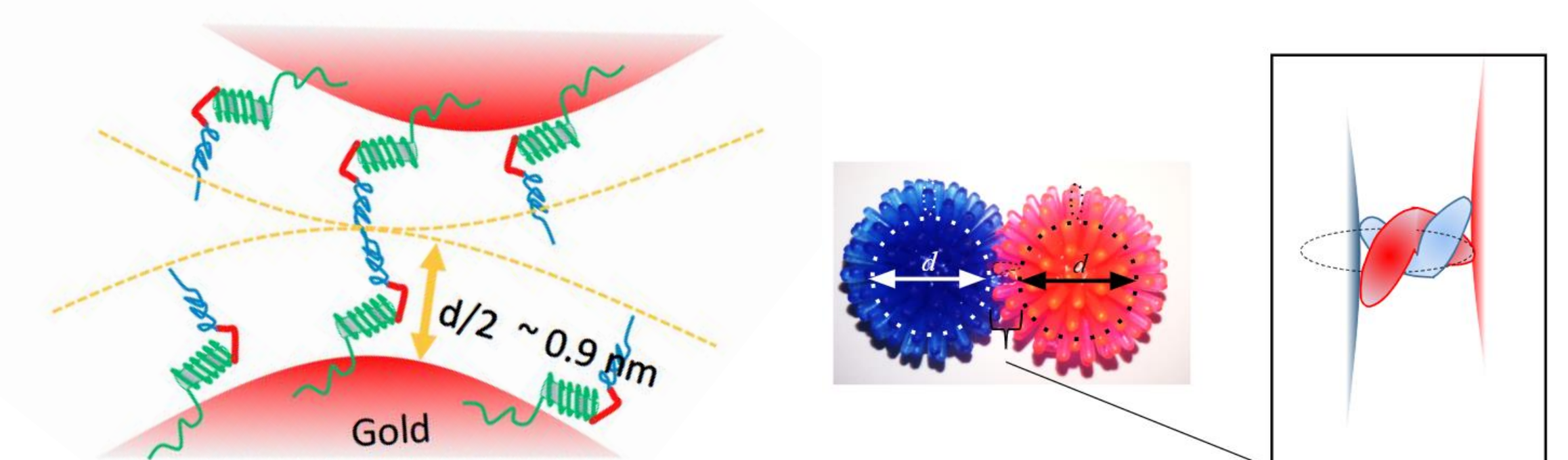
Examples of the distribution plot for d . The distribution was analyzed by a Gaussian model.



The list of the average number of gold colloid in each aggregate and average sepaion distance d (nm)

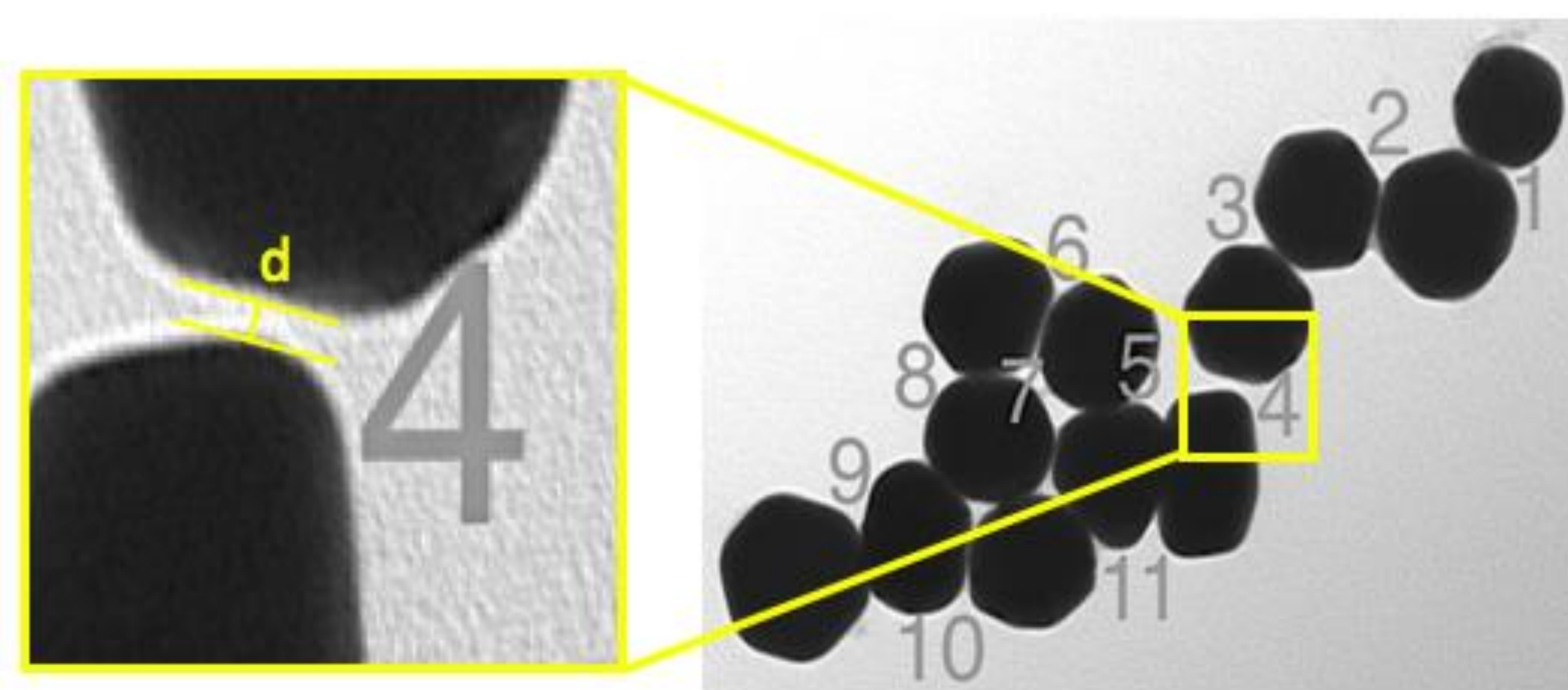
| pH and Size of Gold Particles | | Average Number of Colloids in a Cluster | d (nm) |
|-------------------------------|--------|---|--------------------|
| 10 nm | Acidic | 25.18 | 2.22 ± 0.58 nm |
| | Basic | - | - |
| 30 nm | Acidic | 122.56 | 1.88 ± 0.72 nm |
| | Basic | - | - |
| 60 nm | Acidic | 38.88 | 1.95 ± 0.67 nm |
| | Basic | 12.50 | 1.75 ± 0.36 nm |
| 80 nm | Acidic | 17.28 | 2.13 ± 0.52 nm |
| | Basic | 7.13 | 1.52 ± 0.36 nm |

CONCLUSIONS



This study extracted that the distance between two adjacent gold nano-particle is approximately 2 nm no matter the size of the gold colloid varies. On-going another project of our group disclosed that the orientation of the peptide is a "spiking - out". Considering that the dimension of longer axis is *c.a.* 9.2 nm, two peptides must face by "entangling" each other resulting in the distance between gold surface to be around 2 nm.

ANALYSIS



In certain aggregates, especially pH 4, we noticed spacing between adjacent gold nanoparticles. The space represents the region where peptide-peptide networking are taking place. Due to the limit in resolution by TEM, the peptide networking is not resolved. However, this spacing provides the best information of the dimension of the peptides to form networking at the interfacial environment.

The ImageJ program was used to measure the distance between adjacent gold particles. Each space between the gold particles were given a number of pixels, and corresponding actual distance per pixel was converted to the adjacent distance, d . The spaces between adjacent colloids were manually conducted in image for each size of $\beta 2m$ -coated gold particle. Also, the number of gold particles in each aggregate was individually counted.